



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <b>(54) Title:</b> COMPOSITION AND METHOD<br><br><b>(57) Abstract</b><br><br>Compositions that are sprayable or that are in the form of discrete particles and that contain a lipid and a biological-<br>ly active compound in the form of a micronised powder combine a high initial entrapment of the active compound in the<br>membrane lipid with sustained release at the site of application as indicated by in-vitro and in-vivo tests. In a first form<br>pro-liposomes are prepared by spraying under pressure through a nozzle a single composition comprising at least one vol-<br>atile liquid propellant, at least one membrane lipid that is at least partly dissolved or dispersed in the propellant and at<br>least one biologically active compound that is present in dispersion in the propellant and/or the lipid, the composition be-<br>ing free from other solvent for the lipid. In a second form the membrane lipid and the biologically active compound are<br>minor components of micronised solid particles whose major component is a physiologically acceptable solid carrier. |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

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COMPOSITION AND METHOD

This invention relates primarily to a composition based on a membrane lipid and at least one biologically active compound in the form of a micronised powder and to  
5 a method of making a progenator of liposomes or pro-liposome in the form of an aerosol or discrete particles.

Membrane lipids are lipids which form bilayers with water; they are chiefly phospholipids such as lecithin  
10 and related materials such as glycolipids. Phospholipid vesicles are also known as liposomes. In addition to naturally occurring membrane lipids, synthetic bilayer-forming materials may also form similar vesicles known as niosomes. According to the general nomenclature, all  
15 types of lipid bilayers surrounding an aqueous space are generally known as liposomes which term shall include niosomes. The promise held out by liposomes as a means of delivering and targeting drugs has, not surprisingly, prompted intensive research into this subject.

20 Our European Patent Application No. 85301602 describes pro-liposome compositions that spontaneously form vesicles or liposomes in the presence of excess water and that may be presented inter-alia in sprayable form. Thus there are provided aerosol compositions comprising in  
25 a volatile liquid propellant:-

- (a) at least one membrane lipid;
  - (b) at least one water-miscible liquid which is a solvent for the lipid; and
  - (c) up to 40%, by weight on the combined weights  
30 of a), b) and c), of water;
- the proportion by weight of a) to b) being from 40:1 to 1:20.

This invention is based on the realisation that sprayable compositions or compositions in the form of  
35 discrete particles containing a bilayer lipid and a biologically active compound in the form of a micronised powder combine high initial entrapment of the active

compound in the lipid with sustained release at the site of application.

#### AEROSOL COMPOSITIONS

It has been found that pro-liposomes may be made in the form of aerosols of at least one membrane lipid and at least one biologically active compound, the aerosol being free from other solvents. Further advantages of this type of composition include freedom from unnecessary solvents or water, more control over particle size and improved stability.

A preferred method of forming the pro-liposome aerosols is by spraying under pressure a single composition which comprises at least one volatile liquid propellant, at least one membrane lipid that is at least partly dissolved or dispersed in the propellant and at least one biologically active compound that is present as a micronised powder dispersed in the propellant and/or the lipid, the composition being free from other solvent for the drug.

The invention further provides a sprayable pro-liposome composition comprising at least one volatile liquid propellant, at least one membrane lipid that is at least partly dissolved or dispersed in the propellant and at least one biologically active compound that is present as a micronised powder dispersed in the propellant and/or the lipid, the composition being free from other solvent for the lipid.

It is well known that biologically active compounds can be incorporated into liposomes partly to protect and partly to enhance their activity as mentioned in our European Patent Specification No. A-0158441. One of the features of this invention is the relative ease with which high levels of drug or other biologically active compound can be associated with liposomes.

It has been demonstrated by in-vitro tests that pro-liposome compositions according to the present invention not only enable high initial entrapment of drug, but

surprisingly, also provide for sustained release of the drug at the site of application. It has been found quite unexpectedly that compositions according to the present invention are superior to previously known liposome and  
5 pro-liposome aerosol compositions in prolonging the action of drugs at the site of action. Accordingly the compositions of (or made according to) the invention are particularly suitable for bronchial, pulmonary, and nasal inhalation treatments. They are also suitable for oro-  
10 pharyngeal and topical applications where prolonged effects are desirable to minimise frequency of dosage. Combinations of lipid and drug incorporated into the composition will preferably have the proper electrical charges. Most naturally occurring phospholipids are  
15 negatively charged. Some, especially those that are electrically neutral, may require addition of minor amounts of compatible organic materials that can confer the required charge on them. A negative charge can be imparted to the lipid material by adding, for example,  
20 phosphatidic acid or dicetyl phosphate, and a positive charge can be imparted by adding an amine such as stearylamine.

The proportion of lipid in the composition is desirably as small as possible (in order to minimise the  
25 risk of clogging the orifices of the spray canister) but concomitant with obtaining the optimum level of drug association. The weight ratio of drug to lipid will depend on the activity of the drug and may typically be from 1:0.5 to 1:25, more usually 1:1 to 1:24 and  
30 preferably from 1:6 to 1:12. For highly potent drugs, the appropriate drug/lipid ratio may be above 1:25.

The volatile liquid propellant used in the sprayable compositions of the invention may be butane or dimethylether, in circumstances where its use is  
35 permitted, but is preferably a perhalocarbon, particularly Arcton 11 ( $\text{CClF}_3$ ), Arcton 12 ( $\text{CCl}_2\text{F}_2$ ) or Arcton 114 ( $\text{C}_2\text{Cl}_2\text{F}_4$ ). The propellant generally

constitutes from 75% to 99%, usually from 87% to 97% by weight of the overall composition.

Suitable membrane lipids are phospholipids, for example, phosphatidyl choline, such as soy lecithin and egg yolk lecithin and synthetic lecithins, e.g. di-palmitoyl phosphatidyl choline. Other materials such as glycolipids may be used. Other bilayer-forming materials that can be used include long-chain dialkyl dimethyl ammonium compounds, for example di-stearyl dimethyl ammonium compounds such as di-stearyl dimethyl ammonium chloride, di-tallow dimethyl ammonium compounds such as di-tallow dimethyl ammonium chloride and mono- and dialkyl polyoxyethylene derivatives, although possible irritancy may limit their use to non-inhalation applications. In this text, the generic term lipid is used to describe all such bilayer-forming compounds.

Most biologically active compounds can be incorporated into the sprayable composition. In most instances the biologically active compounds should be insoluble or only sparingly soluble either in the lipid or in the propellant. Accordingly they are incorporated into the composition in the form of micronised powders of mean particle size in the range of from 0.5 micron to 8 microns, preferably from 1 micron to 5 microns.

On being sprayed, e.g. from an aerosol container, the propellant preferentially volatilises, leaving an aerosol of the remaining components as a pro-liposome composition in the form of droplets of a size determined by the spray nozzle and internal pressure and preferably below 8 microns. On contacting water, these droplets form a dispersion of liposomes which constitute very effective drug carriers.

#### POWDER COMPOSITIONS

A second method of making the pro-liposome aerosol is from micronised solid particles whose major component is a physiologically acceptable solid carrier and whose minor components are the biologically active compound in

particulate dispersion in the lipid. Glucose or lactose are suitable solid carriers and being water-soluble, they dissolve on contact with a moist surface such as a biological membrane. However, hydrophillic materials such as polysaccharides (dextrans and starches), polymers (polyethylene), proteins (albumen, casein) and soluble collagen may be suitable for non-inhalation applications as long as they are free-flowing micronised powders, and compatible with the lipid.

10 The weight ratio of membrane lipid to carrier is suitably from 1:1 to 1:25, preferably from 1:5 to 1:20. The ratio of drug to lipid is as before. The powder aerosol may be formed by vibrating or propelling the particles from a capsule (as in a spinhaler type device)  
15 into a moving airstream.

It has been found that high levels of entrapment and improved in-vitro release profiles are obtained when the biologically active compound is in micronised dispersion in the lipid. Dispersion/suspension of the drug is  
20 assisted if the lipid is first dissolved in a volatile solvent which is not a solvent for the drug. Perhalocarbons which are conventionally used as propellants, particularly Arcton 11, are suitable because they are inert and are usually poor solvents for most  
25 biologically active compounds. However, depending on the properties of the powder and the drug, other lipid solvents eg. chloroform or dimethyl ether may be used. The solid carrier must not be soluble in the solvent. Alcohol or methanol would be less suitable if the drug is  
30 substantially soluble.

The solvent is evaporated off completely either before or after addition of the solid carrier. The resulting powder composition comprises a uniform mixture of solid particles whose major component is a  
35 physiologically acceptable solid carrier and whose minor components are the biologically active compound in particulate dispersion in the lipid.

In contrast, the powder compositions described by Payne et al (Journal of Pharmaceutical Sciences, Vol.75, No.4, April 1986) require the drug to be in solution in the lipid. A 2:1-methanol/chloroform solution of the drug is loaded onto the powder under carefully controlled conditions. It is reasonable to assume that the solid carrier would be evenly loaded with a coating of lipid and drug in solution. They confined their studies to coarse powders in the range 75 to 600 microns and concluded that their pro-liposome system was ideally suited for so-called lipophilic actives.

We have found that this method gives rise to compositions which may have high initial association (60% - 70%). However, our in-vitro studies have indicated that they do not retain the biologically active compound, whose release profile is quite different, and characterised by uncontrolled release of the active compound into the aqueous phase.

The invention will be illustrated by the following examples.

#### EXAMPLE I

##### Sprayable compositions and entrapment of Fenoterol therein

The following sprayable compositions were made up using soya bean lecithin (SBL), a 23:54:23 parts by weight mixture of Arcton 114, Arcton 12 and Arcton 11 as propellant and micronised fenoterol as its free base or hydrobromide as biologically active compound.

The lipid was initially dissolved in a small quantity of Arcton 11. Micronised fenoterol was dispersed in this solution. The rest of the propellant mixture was added to the suspension. The composition was filled into 20 ml aerosol containers fitted with metered heads delivering a nominal 65 µl.

The samples were used to form pre-liposome aerosols that were allowed to form liposomes by contact with excess water. The % of fenoterol associated with each liposome dispersion was evaluated by the procedure described below.



The aerosol pro-liposome compositions of samples 1 to 5 were sprayed from canisters which contained no dip tube into a phosphate buffer solution (pH7). One gram of propellant/drug/lipid pro-liposome was sprayed into 50 ml of buffer contained in a 250 ml flask adapted to receive the inverted aerosol container above. Liposome suspensions were formed spontaneously as the aerosol mist encountered the buffer solution. The flask was gently swirled to contact the suspended aerosol with the buffer. This process was repeated until the aerosol canister had been drained of one gram of its contents. Fenoterol was assayed as follows. The fenoterol/liposome suspension was centrifuged at 180,000 g for 90 minutes. The supernatant was assayed for free drug by measuring absorbance at 276 nm. The liposomal pellet containing trapped fenoterol was resuspended in 1% nitric acid to release bound drug and assayed as before. The percentage of drug associated with the liposomes was calculated accordingly.

The entrapment results for each of the five samples are indicated in Table I below.

TABLE I

|               |                   |             |            | % SBL Associated |                   |
|---------------|-------------------|-------------|------------|------------------|-------------------|
| <u>Sample</u> | <u>Propellant</u> | <u>Drug</u> | <u>SBL</u> | <u>Initial</u>   | <u>Entrapment</u> |
| No            | Wt(g)             | Wt(g)       | Wt(Mg)     | HBr              | Base              |
| 25 1          | 9                 | 50          | 50         | 15               | 15                |
| 2             | 9                 | 50          | 150        | 42               | 35                |
| 3             | 9                 | 50          | 300        | 60               | 45                |
| 4             | 9                 | 50          | 600        | 75               | 57                |
| 5             | 9                 | 50          | 1200       | 85               | 72                |

30

EXAMPLE II

Investigation of the effects of lipid-drug ratio on sustained release properties in-vitro

Factors relevant to the sustained release of fenoterol in-vitro were investigated as follows. Fixed weights of aerosol were sprayed into a large conical flask containing 50 ml of phosphate buffer. The flask was

vented at frequent intervals to prevent the build up pressure from vapourised propellant. The final concentration of fenoterol hydrobromide in the test suspension was normally about 0.5 mg/ml but measurements 5 can be made using drug concentrations as low as 0.05 mg/ml. The test suspension was incubated at 37°C. Samples were removed at intervals and centrifuged for 30 min at 175,000xg to pellet the lipid fraction. The concentration of fenoterol in the supernatant fraction was 10 then measured by HPLC. The percentage of drug retained in the lipid fraction was calculated by comparison with the total fenoterol content of a similar non-centrifuged sample in which the lipid had been solvated in excess methanol. This data was then used to plot delayed release 15 profiles of the different samples.

The effects of altering the ratio of standard lipid I (95% pure soya bean lecithin) to fenoterol hydrobromide on the release profiles of our samples are illustrated in Figure 1 in which percentage retention of fenoterol is 20 plotted as a function of time in hours for so-called Type I aerosols prepared from standard lipid I with lipid:drug ratios of 12:1 (circles), 6:1 (squares) and 3:1 (triangles). The descending form of the curves denotes an initial high association between the fenoterol and the 25 lipid phase, a subsequent slow release of part of the fenoterol into the aqueous medium and an eventual steady state determined by the partition coefficient between aqueous and lipid phases. The main effect of increased lipid content, apart from changing the final equilibrium 30 position, was to alter the proportion of drug released within the first 30 min after spraying. Unfortunately, the time involved in sample preparation and centrifugation precluded an analysis of the release characteristics over this period. However, the results suggested that there 35 was an initial very rapid release from solvated droplets followed by a slower release over a period about three hours before equilibrium was reached. This was more

clearly demonstrated in the three semi-logarithmic plots of percentage retention against time for the different samples presented in Figure 2.

### EXAMPLE III

#### 5 Effect of Lipid Modifiers

The inclusion of materials that might be expected to affect the lipid phase was investigated in-vitro. The effects of including cholesterol (33 mol %) and the saturated triglyceride glycerol tripalmitate (33 wt. %) in 10 12:1 lipid/drug formulations was investigated by determining percentage retention of fenoterol as a function of time for Type I aerosols of 12:1 drug/lipid ratio made from standard lipid, mixtures of standard lipid with cholesterol 33 mol %, standard lipid and glycerol 15 tripalmitate 33 wt % and standard lipid and SPAN 40 (10 wt %). The effects of adding the surfactant SPAN 40 (10 wt. %) which might be expected to hasten hydration are shown for comparison. In practice, all the modifiers appeared to bring about increased rates of drug release in-vitro. 20 The precise reasons for this are not clear. The most probable explanation, however, is that their different solubilities in the aerosol propellant resulted in the precipitation of the lipid as a phase-separated rather than a homogeneous mixture and that this leads to changes 25 in the structural characteristics in the lipid droplets. However, in certain situations the use of standard modifiers need not be excluded.

### EXAMPLE IV

#### Use of Different Lipids

30 The effects on sustained release of fenoterol of using two grades of soya bean lecithin (standard lipid I and II); purified egg yolk lecithin (EYL), purified hydrogenated lecithin, EYL + phosphatidyl choline, and EYL + phosphatidyl serine were investigated using the 35 techniques outlined above. Fenoterol was released from such preparations extremely quickly. This extreme leakiness could be associated with the fact that the

hydrogenated lipids tend to be in the gel rather than the liquid crystalline state at 37°C. However, addition of cholesterol, which tends to disrupt gel-phase lipid, led to little or no-improvement in the sustained release capacities of the preparations suggesting that other factors may be influencing their properties.

A particularly striking feature of the measurements performed using hydrogenated lipids is the extremely low interaction between the lipid and the drug. Earlier experiments have suggested that the higher the purity of the lipid, the less strongly bound the drug. In order to test whether the lack of binding of the hydrogenated lipids was associated with their hydrogenation or their high purity, we performed a series of experiments using highly purified lecithin (Lipid Products, Grade 1) standard lipid I (95% pure soy-bean lecithin) and another grade of partially purified lecithin (Lipid II). It was clear from measurements obtained that the Lipid I and Lipid II preparations performed better in terms of delayed release than the highly purified lipid. This result probably reflected the presence in the samples of species providing extra drug binding sites. The most likely candidates for such a species were the acidic lipids phosphatidyl-ethanolamine and phosphatidylserine. We therefore prepared mixtures of purified lecithin together with 25 mol% of these lipids. Delayed release profiles of these samples showed that inclusion of the acidic lipids led, in both cases, to an increase in drug binding resulting in higher final equilibrium levels but the effects were more marked for phosphatidylserine than for phosphatidylethanolamine. The presence of these lipids, however, did not seem to delay the initial fast release characteristics of the purified lipid. This result was in agreement with studies using negative amphipaths such as dicetyl phosphate. The inclusion of such materials appeared to increase the partition of fenoterol into the lipid phase giving rise to high initial association but

also tended to increase leakage rates.

#### EXAMPLE V

##### Structure of the Lipid Droplets Delivered by the Aerosols

The compositions readily formed liposomes when  
5 sprayed onto an aqueous surface. Examination by freeze-  
fracture electron microscopy revealed a mixture of  
partially hydrated lipid aggregates and liposomes. The  
lipid aggregates appeared to be composed of lipid in the  
process of hydration and were likely to reorganise into  
10 liposomes on standing. Figure 3 which is on a scale of 1  
cm equals 1.1  $\mu$ M shows a typical field of liposomal  
structures formed on spraying a Type I aerosol containing  
fenoterol into water. The field contained liposomes (L)  
and partially hydrated liquid aggregates (PHA). The EMs  
15 shown in Figure 3 clearly showed liposomes in the process  
of budding-off from such an aggregate. The possibility  
that some solid fenoterol particles were associated with  
the lipid aggregates in Figure 3 could not be excluded.  
These might have acted as nuclei for the formation of  
20 lipid aggregates and hence functioned as reservoir of  
solid drug which would gradually have released the drug  
over a period of time. We believe that at the site of  
application (in-vivo) the majority of particles initially  
deposit as PHA followed by a gradual rather than an  
25 instantaneous process of conversion into liposomes.

#### EXAMPLE VI

##### Spray Characteristics

For inhalation applications, it is desirable that  
the majority of the droplets should be predominantly in  
30 the 2 to 7 micron range. Only particles in this range are  
capable of reaching the lower regions of the lung.  
Measurements of the droplet size from two compositions  
with lipid/drug ratios 6:1 and 12:1 were carried out in a  
Laser Beam Droplet & Particle Size Analyser (Malvern  
35 Instruments). Figures 4a and 4b show the effect of  
lipid/drug ratios on droplet size. The mean proportion of  
droplets of particle diameter below 7.5  $\mu$  is seen to

decrease from a cumulative value of about 65% to 55% as the lipid/drug ratio increases from 6:1 (Figure 4a) to 12:1 (Figure 4b).

In order to determine the proportion of the drug dose delivered within the respirable range, measurements were made using a cascade impactor (C.F. Casell and Co., London). This type of cascade impactor consists essentially of a system of four air-jets impinging in series on glass discs. The successive jets are progressively finer, so that the speed and, therefore, the efficiency of impaction of particles on to the discs increases from jet to jet, when air is drawn through at a steady rate. A size-grading thereby results which is used to assess the samples.

The drug collected on Plate 1 (particle range 7 - 20  $\mu\text{m}$  diameter) was assumed to be contained within droplets that would be too large to be inhaled. Droplets in this size range would normally be expected to impact on the throat and be ingested. Drug collected on Plate 4 (particle range up to 0.7  $\mu\text{m}$  diameter) was assumed to be contained in drops so small that they would probably be immediately exhaled. Only drug collected on Plates 2 and 3 (particle range 0.7 - 7.0  $\mu\text{m}$ ) was considered to lie within the respirable range.

A series of sprays (12:1 lipid/drug ratio) were prepared, which had vapour pressures ranging from 41 psi to 64 psi, the highest pressure at which samples could easily be prepared (by using different proportions of Arcton 11 and 12). These formulations were sprayed into the cascade impactor through which an air-flow of 17.5 litres per minute was being drawn. Each formulation was sprayed through a 0.75 mm nozzle and also through one with a finer orifice (0.5 mm). The amounts of drug impacting on Plates 3 and 4 were determined by HPLC. The results of these measurements are presented in Table II.

TABLE II

RESPIRABLE DOSE (ug) DELIVERED PER ACTUATION BY TYPE 1  
(12:1) AEROSOLS FITTED WITH DIFFERENT NOZZLES MEASURED AT  
DIFFERENT PROPELLANT VAPOUR PRESSURES

| Sample                                | <u>Vapour Pressure (psi)</u> |      |      |      |      |
|---------------------------------------|------------------------------|------|------|------|------|
|                                       | 41                           | 47   | 51   | 55   | 60   |
| Standard Nozzle<br>(at least 0.75 mm) | 12.4                         | 8.7  | 14.9 | 10.6 | 13.1 |
| Small Nozzle<br>(0.5 mm)              | 22.6                         | 16.4 | 20.7 | 23.7 | 25.8 |

They indicate that changes in vapour pressure have little effect on the fraction of the original dose delivered in the respirable range using the same nozzle size. Increasing the vapour pressure of the samples did, however, decrease the 7 - 20 micron component. The biggest improvement in drug delivery was attained using the smaller nozzle. This was evident at all the vapour pressures employed.

On the basis of these measurements, applicants are of the opinion that by selecting an appropriate combination of vapour pressure and nozzle size the delivery of pro-liposome droplets within the respirable range can be maximised.

EXAMPLE VIIIn-vitro Sustained Release Comparisons

The sustained release properties of the compositions according to the invention have so far been demonstrated under in-vitro conditions. The data obtained provide good evidence that the present invention provides for the sustained release of a biologically active compound. It has also been demonstrated that the compositions of the invention are at least as effective as pro-liposome aerosol compositions disclosed in our earlier publication EP-A-0158441 in delaying the release of a biologically active compound. A composition according to the present

invention, designated Type I, was tested against two other formulations (Type II and Type III) disclosed in publication EP-A-0158441.

TYPE I: A composition according to the present invention where the micronised drug is dispersed uniformly in a blend of Arcton 11, 12 and 114, having dissolved therein a lipid.

TYPE II: In this example, the biologically active compound was dissolved in a pro-liposome composition containing a small quantity of alcohol as solvent for the biologically active compound. The propellant was the same as in Type I.

TYPE III: In this example the drug was dissolved in a pro-liposome composition which contained both alcohol and water. The propellant was the same as in Types I and II.

Liposomal suspensions of all three formulations were formed in-situ by spraying into an aqueous reservoir as previously described.

In this experiment, the release of drug from the liposomal suspensions was determined in a diffusion cell. The preparation was placed on one side of a 4 cm diameter dialysis membrane in the cell. Buffer was pumped through a narrow space under this membrane at a fixed rate of 2 ml per minute. The solution emerging from the membrane cell was returned to the reservoir for recirculation. The rate of diffusion of the drug through the membrane was monitored by HPLC.

It can be seen from Figure 5 that all three pro-liposome aerosol compositions markedly delayed the release of fenoterol (compared to a control solution of the drug) over a period of 12 hours. Figure 5 shows sustained release profiles of sprayed liposome suspensions prepared from Type I, Type II and Type III aerosols. The aerosol preparations were sprayed into fixed volumes of water to form liposomes and collected as described above. 5 ml aliquots were then placed in a membrane cell for measurement of drug release. Each sample contained 5 mg



ml<sup>-1</sup> fenoterol and 60 mg ml<sup>-1</sup> of lecithin giving a lipid/drug ratio of (12:1). It may, however, be noted that according to the in-vitro model, Type III > I > II in terms of sustained release profiles.

5

EXAMPLE VIIIIn-vivo Sustained Release Comparisons

It was considered desirable that the in-vitro findings should be extrapolated to in-vivo situations. Therefore in order to establish in-vitro/in-vivo  
10 correlation, the following controlled studies were carried out on guinea pigs. Individual guinea pigs were exposed to a histamine aerosol in an inhalation chamber. After a short induction period, acute shortness of breath (dyspnea and tachypnea) was provoked, forcing the animal to lie on  
15 its side. If initial treatment with a bronchodilator (eg. a B<sub>2</sub> sympathomimetic) was administered prior to histamine challenge, the induction time before dyspnea set in may be delayed. The period of induction can therefore be used to evaluate the bronchodilatory effect of a biologically  
20 active compound or formulation. The method is based on data by P. Kallos and W. Pagel (Acta. Meda. Scand. 91 (1937) 292).

In an extension of this method, the time interval between initial treatment with a bronchodilator and  
25 challenge with histamine was progressively increased. The length of the induction period before dyspnea set in gave an indication of the duration of action of the bronchodilator. Therefore, the sustained release properties of various formulations could be further  
30 compared.

Test Procedure: The tests were carried out on male and female Pirbright white guinea pigs each weighing between 250 to 300 gm. Each animal was housed in a separate 2 l glass chamber during the test. At time zero, a fixed dose  
35 of aerosol containing fenoterol was sprayed into the chamber. In a parallel control, another guinea pig was not exposed to any bronchodilator formulation. After an

interval of 120 minutes the two guinea pigs were each challenged with a standard dose of 0.2% histamine. The elapsed time in seconds between administration of 0.2% histamine and the first appearance of dyspnea was recorded. A fresh animal was used each time and each animal was only used once.

In a further series of experiments the time interval between pre-treatment with the bronchodilator sprays and challenge with histamine was increased to 180 minutes. A typical set of results from 2 formulations based on the invention is shown in Table III. It gives the induction period (in seconds) for individual guinea pigs after initial treatment with the spray material containing 400 ug fenoterol in a 6:1 (LH 18) and 12:1 (LH 19), lipid/drug ratio. For comparison, the results from six untreated guinea pigs (control) are shown.

TABLE III  
ELAPSED TIME IN SECONDS BETWEEN EXPOSURE TO FENOTEROL AND  
ONSET OF DYSPNEA

| 20 | <u>After 120 Minutes</u> |      |      | <u>After 180 Minutes</u> |      |
|----|--------------------------|------|------|--------------------------|------|
|    | Control                  | LH18 | LH19 | LH18                     | LH19 |
|    | 84                       | 298  | 185  | 138                      | 98   |
|    | 86                       | 601  | 215  | 140                      | 158  |
|    | 58                       | 163  | 138  | 210                      | 88   |
| 25 | 88                       | 171  | 140  | 208                      | 164  |
|    | 66                       | 185  | 201  | 72                       | 118  |
|    | 86                       | 191  | 208  | 91                       | 300  |

Formulations based on variants of the invention (Type I) were compared against Type II and Type III formulations.

The ranking order of the various formulations based on the scores obtained are set out in Table IV. The statistical method for determining the ranking order of the different formulations was based on the Rang-Summon Test (ref: Lehrman, E.L. Non parametrics: Statistical

Methods based on ranks, San Francisco, Holden-Day 1975).

TABLE IV

## RANKING ORDER OF VARIOUS FORMULATIONS AFTER 120 MINUTES

|    | <u>Formulation</u> | <u>Type</u> | <u>Fenoterol</u> | <u>Lipid/Drug</u> | <u>n</u> | <u>Sum of Scores</u> |
|----|--------------------|-------------|------------------|-------------------|----------|----------------------|
| 5  | LH18               | I           | 400 µg           | 6:1               | 6        | 233.5                |
|    | LH19               | I           | 400 µg           | 12:1              | 6        | 198.00               |
|    | LH 1               | I           | 200 µg           | 12:1              | 6        | 139.67               |
|    | LH 4               | II          | 400 µg           | 6:1               | 12       | 127.67               |
|    | LH 7               | III         | 400 µg           | 6:1               | 6        | 127.67               |
| 10 | LH 9               | II          | 400 µg           | 12:1              | 6        | 101.00               |
|    | LH12               | III         | 400 µg           | 12:1              | 6        | 86.71                |
|    | CONTROL            | NIL         | NIL              | NIL               | 30       | 41.18                |

It can be seen from Table IV that there was broad correlation between the in-vitro and in-vivo data. Type I, Type II and Type III formulations all had sustained release properties compared with a control after 120 minutes.

The prolonged effect of the Type I compositions was particularly evident when the interval between initial treatment and subsequent challenge with histamine was increased to 180 minutes. It would appear that after 180 minutes fenoterol protected in the pro-liposome droplet of the invention was twice as active as the unprotected drug. This is shown in Table V.

TABLE V

## RANKING ORDER OF THE INVENTION WITH 12:1; 6:1; LIPID/DRUG RATIOS AFTER 180 MINUTES

|    | <u>Formulation</u> | <u>Type</u> | <u>Fenoterol</u> | <u>Lipid/Drug</u> | <u>n</u> | <u>Sum of Scores</u> |
|----|--------------------|-------------|------------------|-------------------|----------|----------------------|
|    | LH18               | I           | 400 µg           | 6:1               | 6        | 208.5                |
| 30 | LH19               | I           | 400 µg           | 12:1              | 6        | 190.5                |
|    | UNPROTECTED DRUG   |             |                  |                   | 6        | 106.00               |
|    | CONTROL            |             |                  |                   | 6        | 43.00                |

It also appears that with this invention a

lipid/drug ratio of 6:1 is as effective as 12:1 in providing sustained bronchodilatory effect after 180 minutes in experimental animals.

The following examples demonstrate the high initial entrapment and improved retention of powder compositions according to the present invention.

#### EXAMPLE IX

50 mg of micronised fenoterol HBr was dispersed uniformly in a solution of soy-bean lecithin (600 mg) 95% pure in 2 g of Arcton 11. The Arcton 11 was allowed to flash off, leaving behind a dispersion of fenoterol particles in lipid. To this dispersion, 5 g of micronised lactose was added. The resultant powder composition was tumbled in a ball-mill to ensure uniform mixing.

15

#### EXAMPLE X

In a variation of this method, the lactose was added to the suspension of fenoterol in Arcton 11 with the lipid in solution. The Arcton 11 was flashed off, leaving behind a uniform powder composition.

20 In both examples IX and X, the fenoterol was in solid dispersion in the lipid.

#### EXAMPLE XI (Comparative)

In this example, 50 mg fenoterol was first dissolved in 2 g of ethanol. 600 mg of lipid was solvated in 4 g of Arcton 11. The 2 solutions were combined and loaded gradually onto 5 g of lactose. The solvents were evaporated off in a stream of nitrogen to leave behind a powder composition wherein the drug was in solution in the lipid. This example produced a powder composition that resembled the one described by Payne insofar as that the drug was in solution in the lipid.

30

#### EXAMPLE XII

The three components were separately added to a ball-mill or air jet microniser to obtain the required particle size and dispersion of drug in lipid. In this case, no solvents were required.

35

The measurements for initial entrapment and in-vitro

release of fenoterol from the four examples IX-XII were carried out in similar fashion to the aerosol compositions. Figure 6 shows the release of fenoterol as a function of time for the four formulations:

- 5   ○ Drug in suspension, and lipid dissolved, in Arcton 11. Lactose added, solvents removed by rotary evaporation.
- Drug and lipid in solution in 2:1 Arcton 11/ethanol (V:V) mixture. Lactose added, solvents removed by  
10   rotary evaporation.
- ◇ Drug in suspension in Arcton 11 containing lipid. Arcton 11 evaporated prior to lactose addition.
- △ Lactose, lipid, drug milled in tumble mill with no solvent addition.

15       It can be seen that Examples IX, X and XII which have the drug in suspension in the lipid show much higher initial entrapment than the formulation of Example XI. They also retained the drug much better than the formulation of Example XI, where the drug was in solution  
20 in the lipid. The reverse curvature of Example XI is believed to indicate a different mechanism of release of the fenoterol which becomes rapidly released into the aqueous phase as the lactose dissolves and then returns by partition into the lipid phase.

25       Drug entrapment and release profiles of the powder compositions according to the invention may be affected by lipid/drug ratios in like manner to the aerosol compositions previously described. Alternative lipid materials such as hydrogenated lipids may be used in place  
30 of soy-bean lecithin without interfering with the sustained release properties of the resulting composition. The compositions of Examples IX and X may be micronised further to obtain an average particle size, ideally between 0.5 and 8 microns, for inhalation applications.

Similar release profiles were found for these further micronised compositions.

In all instances the average particle size of the final powder composition should be less than 100 u and 5 preferably below 60 u.

#### EXAMPLE XIII

A spray formulation was sprayed through a nozzle to give a hydrogenated lipid of high melting point without hydrophilic carrier by forming micronised droplets of the 10 drug in suspension in the lipid from which carrier has volatilised. The resulting material was collected.

Of course liposomes may also be formed by adding an excess of water to the pro-liposome powder composition comprising carrier, lipid and drug, the composition being 15 free from other solvent for the lipid. In that case, the powder is simply contacted with excess water without first being dispersed in air. The invention therefore provides a pro-liposome composition wherein a membrane lipid and a particulate biologically active compound are minor 20 components of discrete micronised particles whose major component is a physiologically acceptable solid carrier. The invention also includes making liposomes from a composition as aforesaid by contact with water either in situ or in-vivo. For oral administration the discrete 25 micronised solid particles may be filled into gelatin or other capsules.

The invention further comprises the administration to a human or animal subject of an aerosol composition as aforesaid for the treatment of bronchial, pulmonary and 30 nasal disorders such as asthma, bronchitis, hay fever and the like containing bronchodilators and steroids, antibiotics, anti-histamines or vasoconstrictors. The invention yet further includes the administration of topical preparations where sustained release of the active 35 compound or compounds is desirable, for example in the treatment of psoriasis and inflammatory skin conditions such as eczema with steroids.

## CLAIMS:

1. A method for the preparation of pro-liposomes ("liposomes" being as defined above) which comprises forming discrete particles of at least one membrane lipid and at least one biologically active compound, such particles being free from solvent for the membrane lipid and said biologically active compound being present as discrete micronised particles.
2. A method according to Claim 1, which comprises spraying under pressure through nozzle means a single composition comprising at least one volatile liquid propellant, at least one membrane lipid that is at least partly dissolved or dispersed in the propellant and at least one biologically active compound that is present in dispersion in the propellant and/or the lipid, the composition being free from other solvent for the biologically active compound.
3. The method of Claim 2, wherein the propellant used is a perhalocarbon.
4. The method of Claim 3, wherein the propellant is  $\text{CClF}_3$ ,  $\text{CCl}_2\text{F}_2$  or  $\text{C}_2\text{Cl}_2\text{F}_4$ .
5. The method of Claim 1, wherein the membrane lipid is a natural or hydrogenated lecithin, a glycolipid, a long chain dialkyl ammonium compound or a mixture of any of the aforesaid compounds with a compatible lipophilic material.
6. A method according to Claim 1, wherein ionised biologically active compound has an electrical charge that is of opposite electrical sign to the charge on the lipid material.
7. A method according to Claim 6, wherein an amphipath is present to confer charge on the lipid material.
8. A method according to Claim 1, wherein the biologically active compound is present as particles of average size from 0.5 to 8 microns.
9. A method according to Claim 1, wherein the biologically active compound is salbutamol, terbutaline, orciprenaline, isoprenaline, reproterol, pirbuterol,

butenoside, beclamethasone dipropionate, sodium chromoglycate, fenoterol, ipratropium, betamethasone valerate, rimiterol or ketotifen.

10. A method according to Claim 1, wherein the biologically active compound and lipid are present in a weight ratio of at least 1:0.5.

11. A method according to Claim 9, wherein the drug and lipid are present in a weight ratio of from 1:3 to 1:12.

12. A sprayable pro-liposome composition comprising at least one volatile liquid propellant, at least one bilayer lipid that is at least partly dissolved or dispersed in the propellant and at least one biologically active compound that is present in the propellant and/or the lipid as a dispersed micronised powder, the composition being free from other solvent for the drug.

13. An aerosol canister charged with a single sprayable component that is a composition as claimed in Claim 12.

14. A method according to Claim 1, wherein the bilayer lipid and the biologically active compound are minor components of micronised solid particles whose major component is a physiologically acceptable solid carrier.

15. A method according to Claim 14, wherein the solid carrier is glucose or lactose.

16. A method according to Claim 14, wherein the weight ratio of membrane lipid to carrier is from 1:1 to 1:25.

17. A method according to Claim 14, wherein the weight ratio of biologically active compound to lipid is from 1:0.5 to 1:25.

18. A method according to Claim 14, wherein the weight ratio of biologically active compound to lipid is from 1:6 to 1:20.

19. A method according to Claim 14, wherein the aerosol is formed by shaking or introducing the particles from a container into a moving airstream.

20. A method according to Claim 14, wherein the micronised solid particles of pro-liposome are of average diameter 0.5 to 8 microns.



21. A pharmaceutical composition comprising discrete micronised particles whose minor components are a bilayer lipid and a biologically active compound in dispersion and whose major component is a physiologically acceptable solid carrier.
22. A composition according to Claim 21, wherein the solid carrier is glucose, lactose, a dextran or starch or another micronised powder.
23. A composition according to Claim 21, wherein the weight ratio of membrane lipid to carrier is from 1:1 to 1:25.
24. A composition according to Claim 21, wherein the weight ratio of biologically active compound to lipid is from 1:0.5 to 1:25.
25. A composition according to Claim 21, wherein the weight ratio of biologically active compound to lipid is from 1:6 to 1:20.
26. A composition as claimed in Claim 21, wherein the micronised solid particles of pro-liposome are of average diameter 0.5 to 8 microns.
27. A capsule containing micronised particles of pro-liposome as claimed in Claim 21.
28. A method of making liposomes which comprises mixing a composition as claimed in Claim 21 with excess water.
29. A method as claimed in Claim 28, wherein the mixing step is performed in vitro.
30. A method as claimed in Claim 28, wherein the mixing step is performed in vivo.
31. A pharmaceutical composition comprising discrete micronised solid particles consisting of drug suspended in lipid.

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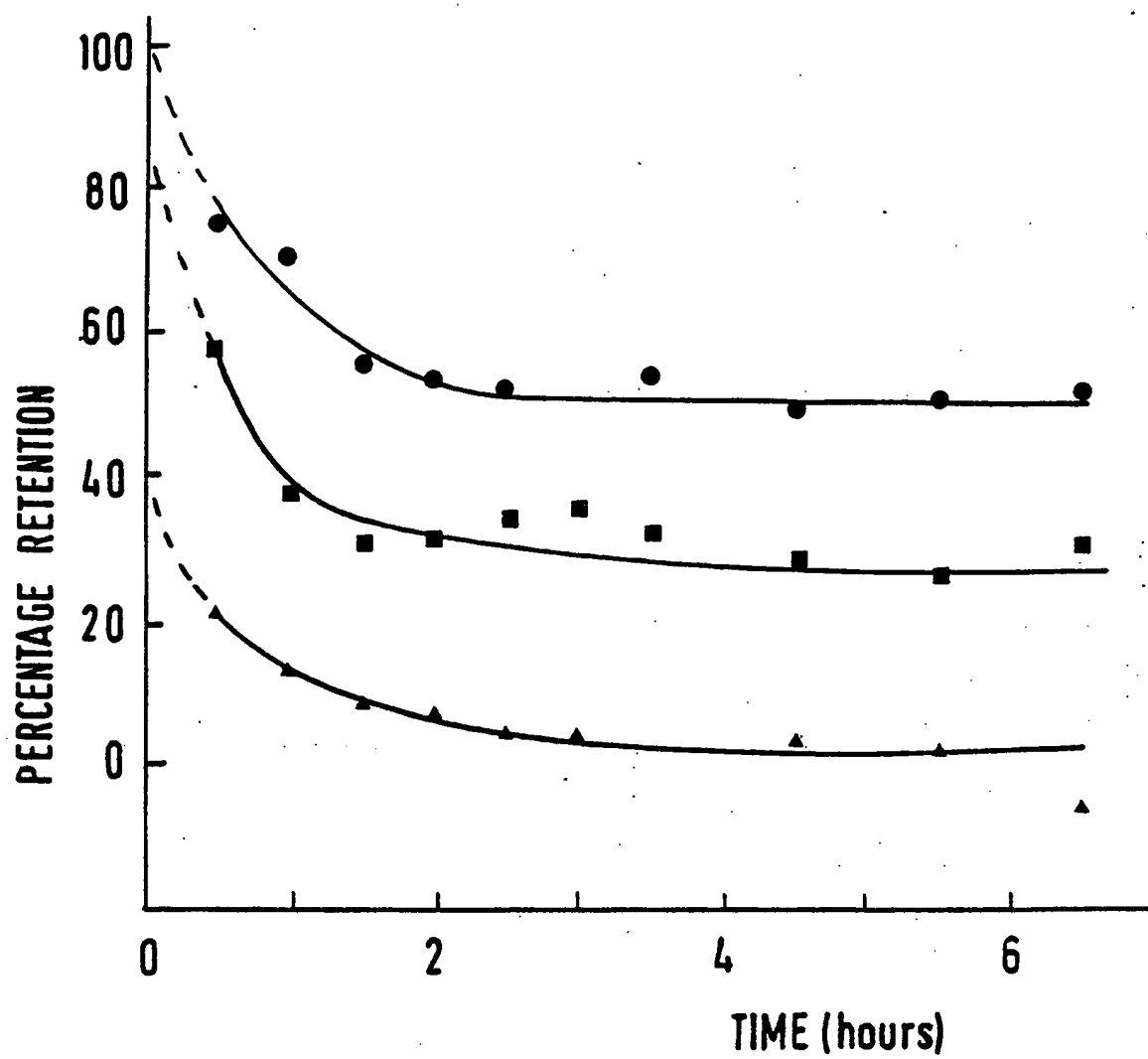
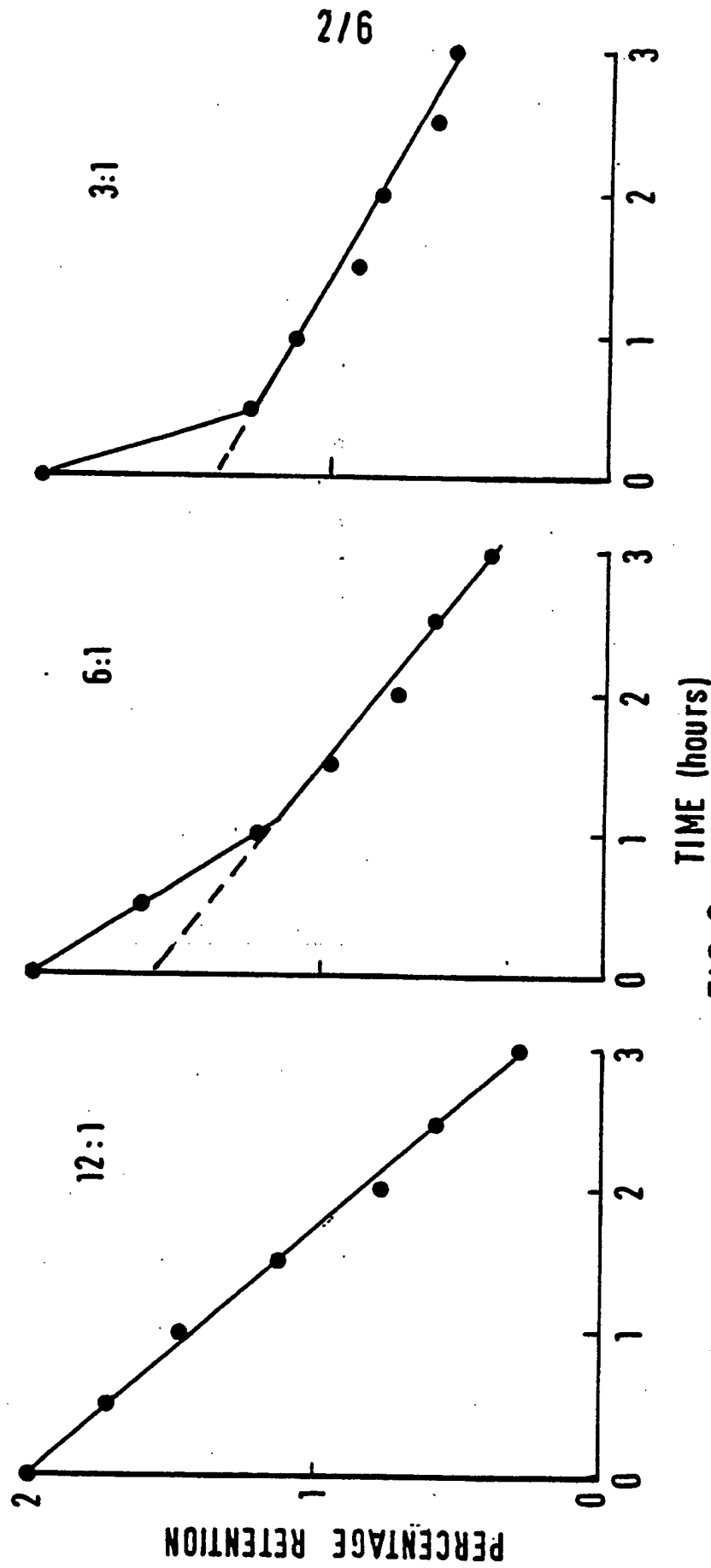


FIG.1



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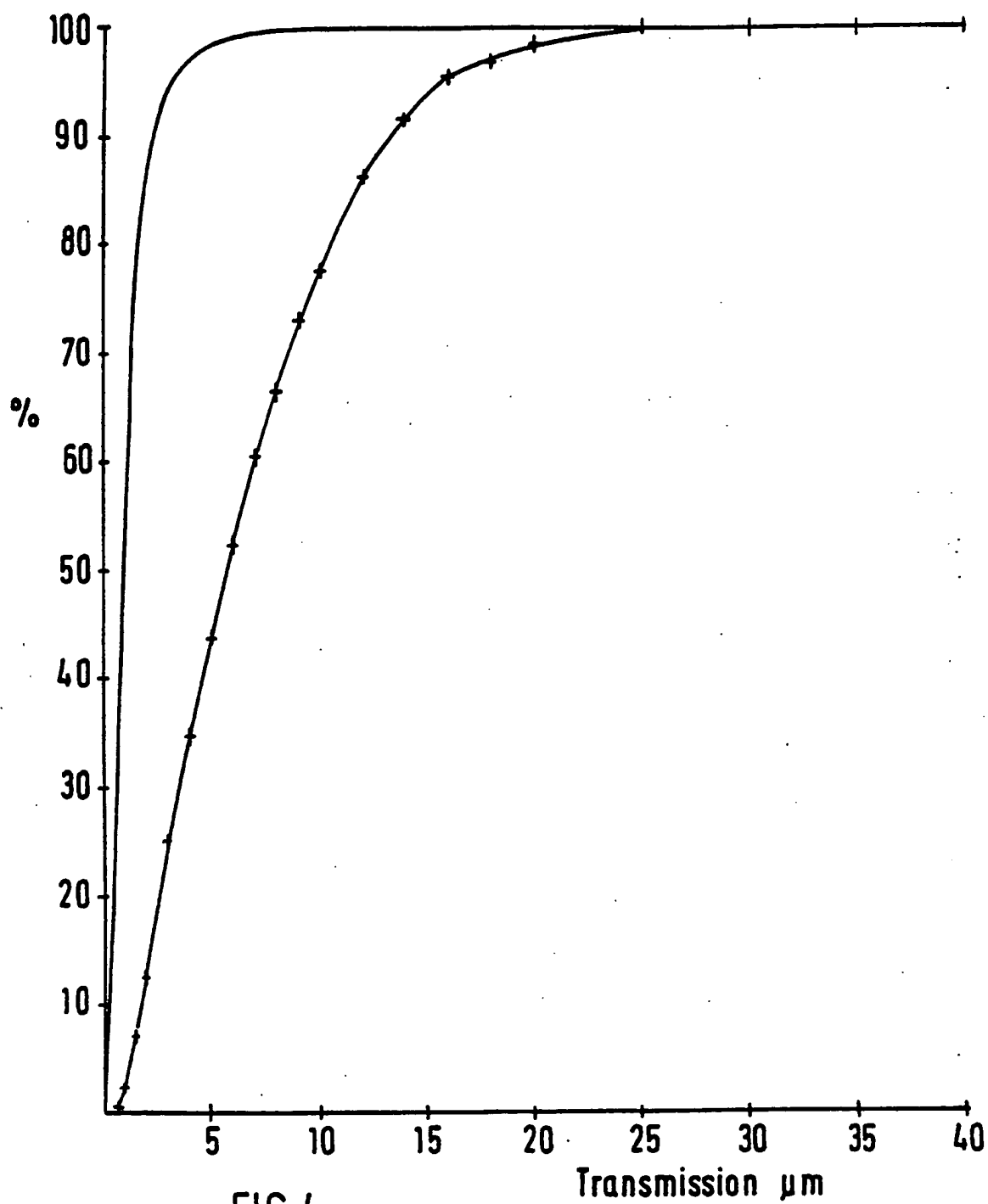
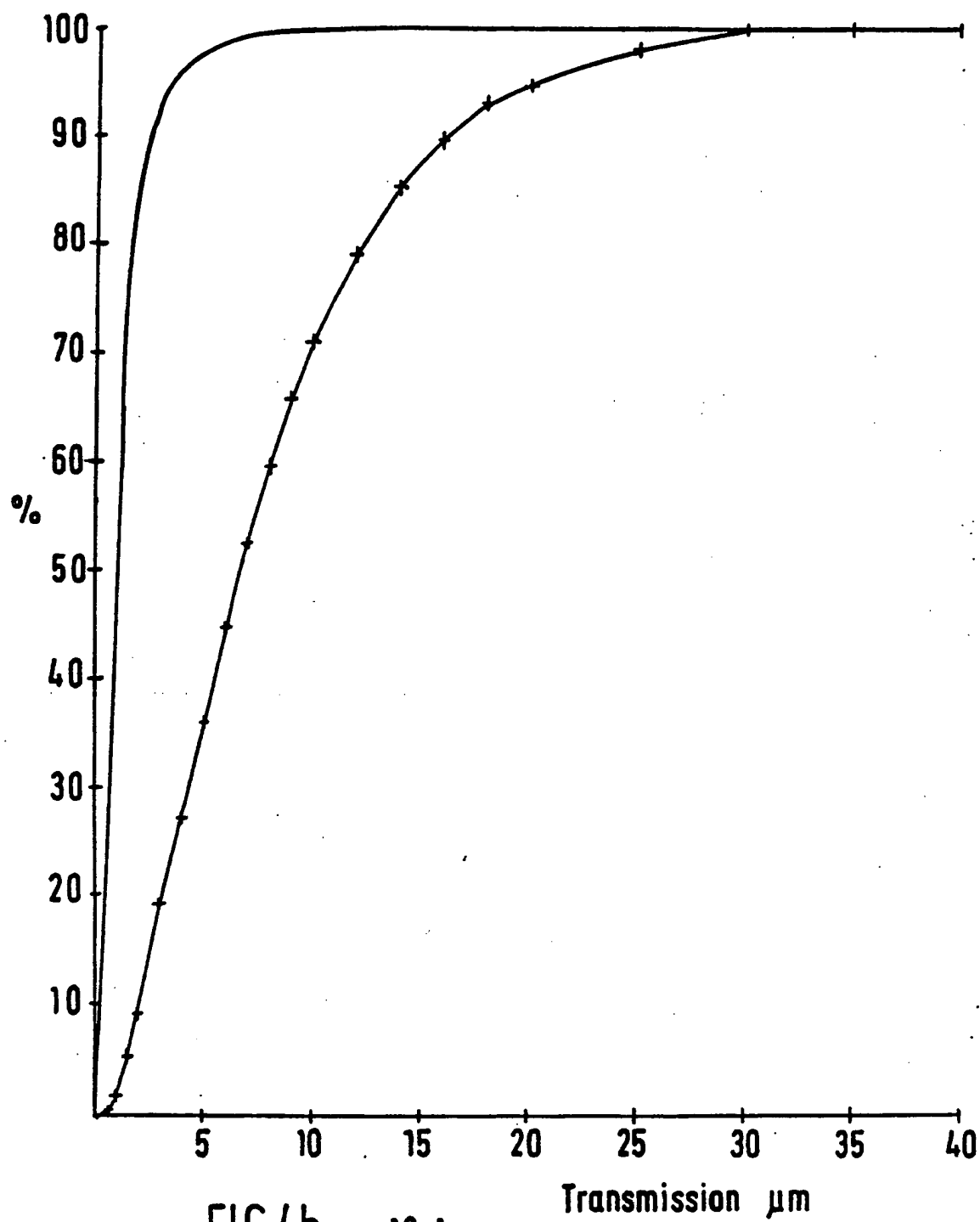


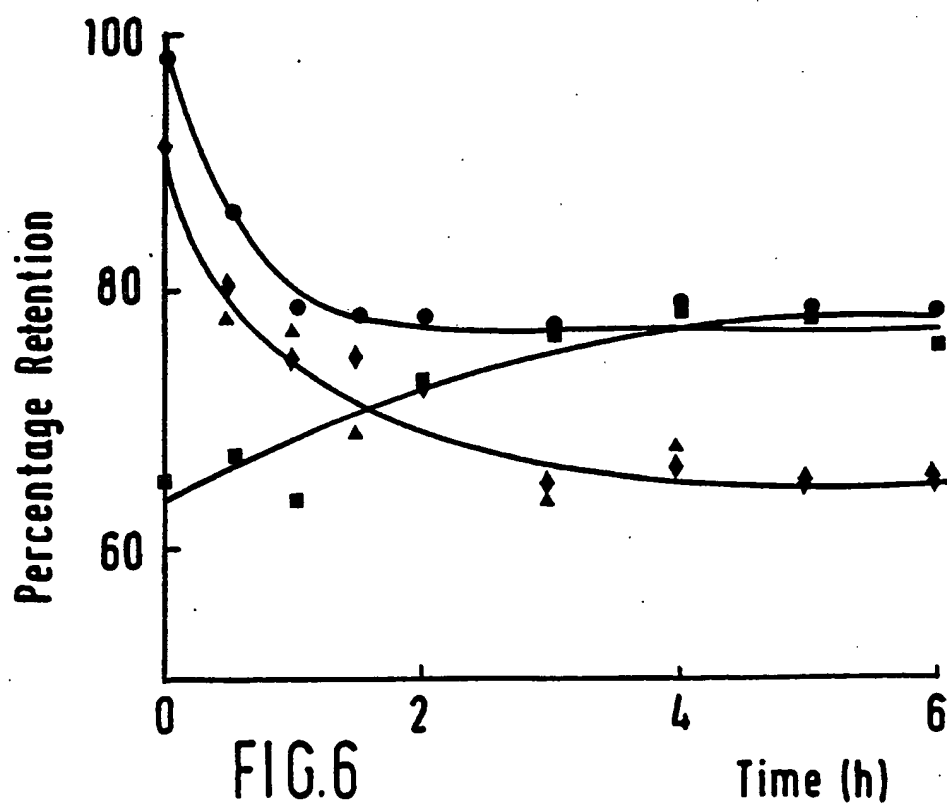
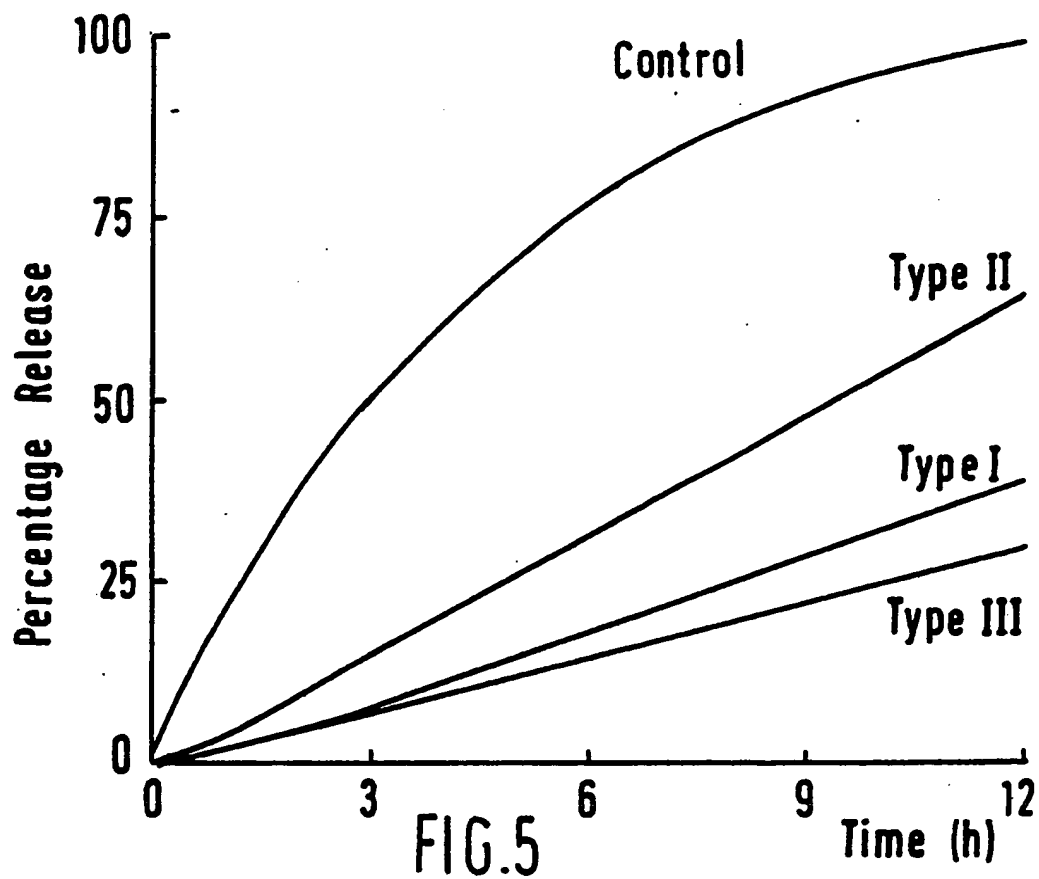
FIG.4a

6:1

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# INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 87/00391

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|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|
| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) *<br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC <sup>4</sup> : A 61 K 9/50; A 61 K 9/12                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                            |                                                     |
| <b>II. FIELDS SEARCHED</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                            |                                                     |
| Minimum Documentation Searched <sup>7</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                            |                                                     |
| Classification System                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Classification Symbols                                                                                                                                                                                     |                                                     |
| IPC <sup>4</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | A 61 K                                                                                                                                                                                                     |                                                     |
| Documentation Searched other than Minimum Documentation<br>to the extent that such documents are included in the fields searched *                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                            |                                                     |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>9</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                            |                                                     |
| Category <sup>9</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>                                                                                             | Relevant to Claim No. <sup>13</sup>                 |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | EP, A, 0087993 (PARFUMS CHRISTIAN DIOR)<br>7 September 1983<br>see page 3, line 33 - page 4, line 10;<br>page 8, line 18 - page 9, line 32;<br>page 15, lines 24-30; page 16, lines<br>25-34; claims<br>-- | 1-31                                                |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | US, A, 3594476 (E.W. MERRIL) 20 July 1971<br>see the whole document<br>--                                                                                                                                  | 1,31                                                |
| E                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | EP, A, 0229561 (MOET-HENNESSY RECHERCHE)<br>22 July 1987<br>see claims 1,13,14<br>-----                                                                                                                    | 1-31                                                |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>14</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div> |                                                                                                                                                                                                            |                                                     |
| <b>IV. CERTIFICATION</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                            |                                                     |
| Date of the Actual Completion of the International Search                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                            | Date of Mailing of this International Search Report |
| 24th September 1987                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                            | - 4 NOV 1987                                        |
| International Searching Authority                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                            | Signature of Authorized Officer                     |
| EUROPEAN PATENT OFFICE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                            | M. VAN MOL                                          |



# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 87/00391 (SA 17467)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 19/10/87

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| Patent document<br>cited in search<br>report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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|                                              |                     | JP-A- 59031707             | 20/02/84            |
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| US-A- 3594476                                | 20/07/71            | None                       |                     |
| EP-A- 0229561                                | 22/07/87            | FR-A- 2591105              | 12/06/87            |

For more details about this annex :  
see Official Journal of the European Patent Office, No. 12/82